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(54) Title: TOPOISOMERASE INHIBITORS FOR PREVENTION OF RESTENOSIS			
(57) Abstract			
A method of inhibiting cellular proliferation associated with a hyperproliferative condition, such as restenosis, is described. The method includes administering a topoisomerase inhibitor. A stent for local administration of the topoisomerase inhibitor is also described.			

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Topoisomerase Inhibitors for Prevention of Restenosis

This application claims the priority of U.S. provisional application Serial No. 60/090,764, filed June 26, 1998, which is incorporated herein by reference.

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Field of the Invention

The present invention relates to a method of inhibiting cellular proliferation in a subject.

10 Background of the Invention

Vascular disease is a leading cause of death and disability in westernized societies. Atherosclerosis is one of the more common forms of vascular disease and leads to insufficient blood supply to critical body organs resulting in heart attack, stroke and kidney failure. Atherosclerosis also causes complications in people suffering from hypertension 15 and diabetes.

Atherosclerosis has been described as a form of vascular injury. A normal vessel wall consists of three reasonably well-defined layers: the intima, the media and the adventitia. The intima lines the lumen of all arteries and is composed of a single continuous layer of endothelial cells. The media consists of only one cell type, the smooth 20 muscle cell, arranged in either a single layer or multiple lamellae. These cells are surrounded by small amounts of collagen and elastic fibers. The outermost layer of the artery is the adventitia, which consists of a loose interwoven admixture of collagen bundles, elastic fibers, smooth muscle cells and fibroblasts (Harrison's PRINCIPLES OF INTERNAL MEDICINE, 12th Edition, McGraw-Hill, Inc., 1991, Chapter 195).

25 While the processes causing atherosclerosis are complex and not completely understood, an underlying pathology to the numerous theories for the cause of atherosclerosis is the abnormal migration and proliferation of medial-smooth muscle cells into the intima (Harrison). The proliferation of the smooth muscle cells in the intima ultimately blocks blood flow and makes vessels abnormally susceptible to local blood 30 clotting.

A similar pathology is also implicated in restenosis, the so-called recurrence of stenosis or artery stricture after corrective surgery. In fact, restenosis has been described as an accelerated atherosclerosis induced by injury (Forrester, J.S., *et al.*, JACC, 17(3):758-769 (1991)). Restenosis results from vascular smooth muscle cell proliferation,

migration and neo-intimal accumulation, due to incompletely understood processes involving regulatory molecules, such as platelet derived growth factor (Ferns, G.A., *et al.*, *Science*, 253:1129 (1991)).

5 Restenosis has been observed to occur after coronary artery bypass surgery, heart transplantation, atherectomy, laser ablation and balloon angioplasty. In particular, restenosis is common after balloon angioplasty, also referred to as percutaneous transluminal coronary angioplasty, which is widely used as a treatment modality in patients with coronary artery disease to reduce lumen obstruction and improve coronary blood flow. It is estimated that between 25-35 % of patients develop restenosis within 1-3 months after 10 balloon coronary angioplasty, necessitating further interventions such as repeat angioplasty or coronary bypass surgery.

Therapies to reduce restenosis have focused on administration of chemotherapeutic agents which either interfere with formation of thrombosis or suppress smooth muscle cell proliferation. Anti-coagulants for suppression of thrombosis include heparin, warfarin, low 15 molecular weight heparin and hirudin (Lovqvist, A., *et al.*, *J. Int. Medicine*, 233:215-116 (1993)). Agents for inhibiting the proliferation of smooth muscle cells include glucocorticoids, angiotensin converting enzyme inhibitors, colchicine, vincristine, actinomycin, low molecular weight heparin, platelet derived growth factor and others (Lovqvist, A., *et al.*). More recently, paclitaxel (TAXOL[®]) has been suggested for use in 20 preventing restenosis (Kinsella, J.L. and Sollott, S. J., U.S. Patent No. 5,616,608, issued April 1, 1997).

However, of all the drugs tested, none have been found to be sufficiently effective, and some, such as colchicine, have been reported to be ineffective (O'Keefe, J.H., *et al.*, *JACC*, 19(7):1597 (1992)).

25

Summary of the Invention

Accordingly, it is an object of the present invention to provide a method of inhibiting cellular proliferation associated with restenosis and atherosclerosis.

In one aspect, the invention includes a method of inhibiting cell proliferation 30 associated with a hyperproliferative condition in a subject by administering to the subject a therapeutically effective amount of a topoisomerase inhibitor.

In one embodiment, the topoisomerase inhibitor is selected from the group consisting of camptothecin, irinotecan and topotecan.

In one embodiment, the hyperproliferative condition is restenosis.

In one embodiment, the method of administration is by local administration of the topoisomerase inhibitor. Local administration of the drug is, in one embodiment, by means of a drug delivery catheter, or in another embodiment, by a guidewire.

5 In another embodiment, the topoisomerase inhibitor is locally administered to the treatment site via a stent carrying the topoisomerase inhibitor. The stent, in one embodiment, is a polymer stent loaded with a topoisomerase inhibitor selected from the group consisting of camptothecin, irinotecan and topotecan.

10 In other embodiments, the stent is a metal stent and the topoisomerase inhibitor is incorporated into a polymer sheath carried on the metal stent, or the metal stent is coated with a synthetic polymer or a biopolymer carrying the topoisomerase inhibitor, or the metal stent includes surface indentations in which the topoisomerase inhibitor is incorporated.

15 In another embodiment, the method includes coadministering a second therapeutic agent with the topoisomerase inhibitor. The second therapeutic agent, in one embodiment, is verapamil, dexamethasone or a microtubule stabilizing agent such as paclitaxel, derivatives of paclitaxel, and colchicine. In another embodiment, the second therapeutic agent is radiation therapy.

In another aspect, the invention includes a method of inhibiting restenosis in a patient by administering to the patient an effective amount of a topoisomerase inhibitor.

20 In another aspect, the invention includes a device for treatment of restenosis, comprising a stent carrying a therapeutically effective amount of a topoisomerase inhibitor.

In one embodiment, the stent is a polymer stent loaded with a topoisomerase inhibitor selected from camptothecin, irinotecan and topotecan.

25 In another embodiment, the stent is a metal stent and the topoisomerase inhibitor is incorporated into a polymer sheath carried on the metal stent. In another embodiment, the metal stent is coated with a synthetic polymer or a biopolymer carrying the topoisomerase inhibitor. In a further embodiment, the metal stent has surface indentations and the topoisomerase inhibitor is incorporated into the pits.

30 The stent, in another embodiment, includes a second therapeutic agent for treatment of restenosis. The second therapeutic agent, in one embodiment, is a microtubule stabilizing agent such as paclitaxel, derivatives of paclitaxel, and colchicine or the agent is verapamil or dexamethasone.

These and other objects and features of the invention will be more fully appreciated when the following detailed description of the invention is read in conjunction with the accompanying drawings.

Brief Description of the Drawings

5 Figs. 1A-1C are illustrations of the basic V-shaped stent used in studies in support of the present invention, where the stent is shown unwound (Fig. 1A), wound to a small diameter for insertion in a vessel (Fig. 1B) and in an open, expanded diameter after placement in a vessel (Fig. 1C); and

10 Figs. 2A-2B are artist-renderings of angiogram images of pig coronary arteries one month after insertion of a camptothecin-loaded stent in accordance with the invention (Fig. 2A) or of a metal, control stent (Fig. 2B).

Detailed Description of the Invention

I. Definitions

15 "Hyperproliferative condition" refers to undesirable cell growth associated with atherosclerosis, restenosis, proliferative vitreoretinopathy and psoriasis. The term is not intended to include cellular hyperproliferation associated with cancerous conditions.

20 "Undesirable cell growth" or "inhibiting undesirable cell growth" refers to unregulated cell division associated with smooth muscle cells or fibroblasts and to the inhibition of such growth.

25 "Topoisomerase inhibitor" refers to any compound that inhibits the action of topoisomerase enzymes, including topoisomerase I and topoisomerase II. Such inhibitors include camptothecin, derivatives and analogs of camptothecin, such as irinotecan, topotecan and 9-amino-camptothecin, etoposide, teniposide, genistein and mitoxantron.

30 "Administering" as referred to herein is intended to include routes of administration which allow the topoisomerase inhibitor to perform its intended function of inhibiting undesirable cell growth. Such administering includes systemic and local or site specific administration by an appropriate route, such as injection (subcutaneous, intravenous, parenteral, intraperitoneal, intrathecal, etc.) oral, inhalation, transdermal, administration by means of a drug delivery catheter, or implantation of a drug-carrying device.

35 "Effective amount" refers to the amount necessary or sufficient to inhibit the undesirable cell growth, *e.g.*, prevent the undesirable cell growth or reduce the existing cell growth. The effective amount can vary depending on factors known to those of skill in the art, such as the type of cell growth, the mode and regimen of administration, the size of the subject, the severity of the cell growth, etc. One of skill in the art would be able to consider such factors and make the determination regarding the effective amount.

"Pharmaceutically acceptable carrier" refers to any substance coadministered with the topoisomerase inhibitor which allows the compound to perform its intended function. Examples of such carriers include solutions, solvents, dispersion media, delay agents, emulsions, microparticles and the like.

5

II. Method of Treatment

In the method of the invention, a topoisomerase inhibitor is administered to a subject at risk of developing or suffering from a hyperproliferative condition. The topoisomerase inhibitor is administered in a therapeutically effective amount by a selected 10 route as will be described.

Preferred topoisomerase inhibitors for use in the method of the invention include camptothecin and analogs thereof. Camptothecin is a pentacyclic alkaloid initially isolated from the wood and bark of *Camptotheca acuminata*, a tree indigenous to China (Wall, M.E. *et al.*, *J. Am. Chem. Soc.*, 94:388 (1966)). Camptothecin exerts its pharmacological 15 effects by irreversibly inhibiting topoisomerase I, an enzyme intimately involved in DNA replication. Methods for the synthesis of camptothecin and camptothecin analogs are known, and are summarized and set forth in U.S. Patent No. 5,244,903, which is herein incorporated by reference in its entirety.

Analogues of camptothecin include SN-38 ((+)-(4S)-4,11-diethyl-4,9-dihydroxy-20 1H-pyrano[3',4':6,7]-indolizino[1,2-b]quinoline-3,14(4H,12H)-dione); 9-aminocamptothecin; topotecan (hycamtin; 9-dimethyl-aminomethyl-10-hydroxycamptothecin); irinotecan (CPT-11; 7-ethyl-10-[4-(1-piperidino)-1-piperidino]-25 carbonyloxy-camptothecin), which is hydrolyzed *in vivo* to SN-38; 7-ethylcamptothecin and its derivatives (Sawada, S. *et al.*, *Chem. Pharm. Bull.*, 41(2):310-313 (1993)); 7-chloromethyl-10,11-methylene-dioxy-camptothecin; and others (SN-22, Kunimoto, T. *et al.*, *J. Pharmacobiodyn.*, 10(3):148-151: (1987); DX-8951f and GG-211 Rothenberg, M.L., *Ann. Oncol.*, 8(9):837-855 (1997)).

Other topoisomerase inhibitors for use in the method of the invention include 4'-(acridinylamino)-methanesulfon-m-aniside (amsacrine) and its analogues, such as N-5-dimethyl-9-(2-methoxy-4-methylsulfonylamino)-phenylamino-4-acridinecarboxamide (CI-30 921, Traganos, F., *et al.*, *Cancer Res.*, 47(2):424-432 (1987)) and methyl-N-[4-9-acridinylamino)-2-methoxyphenyl]carbamate hydrochloride (m-AMCA, Moreland, N., *et al.*, *Eur. J. Cancer*, 33(10):1668-1676 (1997)); 6-N-formylamino-12,13,dihydro-1,11-dihydroxy-13-(beta-D-glucopyranosyl)-5H-indolo[2,3-a]pyrrolo[3,4-c]carbazole-5,7(6H)-

dione (NB-506, Kanzawa, F *et al.*, *Cancer Res.*, 55(13):2806-2813 (1995); etoposide (VP-16, Karato, A., *et al.*, *J. Clin. Oncol.*, 11(10):2030-2035 (1993)); m-AMSA (Nakano, H., *Gan To Kagaku Ryoho*, 18(10):1550-1555 (1991)); 6-[[2-(dimethylamino)-ethyl]amino]-3-hydroxy-7H-indeno[2,1-c]quinolin-7-one dihydrochloride, (TAS-103, Utsugi, T., *et al.*, *Jpn. J. Cancer Res.*, 88(10):992-1002 (1997)); azatoxin (Solary E., *et al.*, *Biochem. Pharmacol.*, 45(12):2249-2456 (1993)); 3-methoxy-11H-pyrido[3',4'-4,5]pyrrolo[3,2-c]quinoline-1,4-dione (AzalQD, Riou, J.F., *et al.*, *Mol. Pharmacol.*, 40(5):699-706 (1991)); anthracenyl-amino acid conjugates (ICRF 506, Meikle, I., *et al.*, *Biochem. Pharmacol.*, 49(12):1747-1757 (1995)); and bis(2,6-dioxopiperazine derivatives (ICRF-154, ICRF-193, ICRF-159 and MST-16, Kizaki, H., *et al.*, *Adv. Enzyme Regul.*, 37:403-423 (1997)).

In one embodiment of the invention, the topoisomerase inhibitor administered is the pharmacologically active enantiomer of a camptothecin analogue having a chiral center. The enantiomer can be resolved from the racemic mixture using techniques known to those of skill in the art.

In another embodiment, the topoisomerase inhibitor is camptothecin or an analogue of camptothecin and is administered in combination with a second therapeutic compound selected from paclitaxel, and derivatives of paclitaxel, calcium channel blockers, such as verapamil, and steroid non-inflammatory agents, such as dexamethasone. When the second therapeutic agent is a calcium channel blocker, it serves to reduce elimination of the first therapeutic agent from the cells. When the second therapeutic agent is an anti-inflammatory agent, it is effective to reduce inflammation at a site of injury.

The therapeutic compounds described above are used in the method of the invention for inhibiting the growth of vascular smooth muscle cells in the vessel. "Inhibiting" as used herein is intended to include reducing, delaying or eliminating undesirable cell growth. With respect to the embodiment of the invention where the undesirable cell growth is associated with restenosis following balloon coronary angioplasty, "reducing" means decreasing the intimal thickening that results from smooth muscle cell proliferation following angioplasty, either in an animal model or in humans. "Delaying" means delaying the time until onset of visible intimal hyperplasia, as observed histologically or by angiographic techniques, following angioplasty. "Eliminating" refers to completely reducing and/or completely delaying intimal hyperplasia in a subject such that sufficient blood flow in the vessel is established and surgical intervention is not necessary.

5 The topoisomerase inhibitors are administered in accordance with the invention by any route which provides effective therapy for the inhibition of restenosis. Such routes include but are not limited to systemic administration of the drug by injection, including bolus, pulsed and continuous administration injected intravenously, subcutaneously, intramuscularly, intraperitoneally, etc. Continuous or delayed release formulations are also contemplated, both for systemic administration and local or site specific administration.

10 One preferred mode of administering the topoisomerase inhibitor is via a drug delivery catheter, such as those described in U.S. Patent Nos. 5,558,642, 5,295,962, 5,171,217 and 5,674,192. Typically, such catheters have a flexible shaft and an inflatable balloon at the distal end of the shaft. The catheter is inserted into a vessel in an un-inflated condition and the balloon is positioned at the site to be treated with the topoisomerase inhibitor. The balloon member is inflated and apertures in the balloon assembly provide drug carried in the catheter to be delivered to the target site. The drug can be carried in solution form, entrapped in microparticles of a physiologically compatible polymer or 15 incorporated into a polymer, such as a hydrogel, which is coated on the balloon region for rapid release of the drug during expansion of the balloon. Catheters such as these provide a convenient way to administer the drug in conjunction with a balloon angioplasty procedure.

20 In other embodiments, the topoisomerase inhibitor is administered to the target site by an infusion catheter or by a drug delivery guidewire. An infusion catheter provides delivery of agents to a target site by placing the tip of the catheter at the site and connecting the catheter to a pump. The tip of the catheter generally includes openings through which the agent is pumped at a desired rate to the target site (U.S. Patent No. 5,720,720). A drug delivery guidewire has been described in U.S. Patent No. 5,569,197, where the guidewire 25 is hollow and has an opening at its distal end for infusion of a drug therethrough.

30 In a preferred embodiment, the topoisomerase inhibitor is administered locally to the site of undesired cell growth in the form of an implanted medical device, such as a stent. Endovascular stents for use following balloon angioplasty are known in the art and described in, for example, U.S. Patent Nos. 5,395,390 (Simon), 4,739,762 (Palma), 5,195,984 (Schatz) and 5,163,952 (Froix).

In one embodiment, the stent is a metal stent. Exemplary biocompatible and nontoxic metals for stents include nickel-titanium alloys, tantalum, and steel. In this embodiment, the topoisomerase inhibitor can be adsorbed onto the stent or incorporated into indentations, *i.e.*, pockets, grooves or pits, formed on the surface of the stent. In

another embodiment, the metal stent is coated with a polymer-drug solution containing the selected topoisomerase inhibitor or drug combination by dipping the stent in the solution or spraying the stent with the solution.

5 The metal stent, in other embodiments, is adapted to carry a polymer member, where the metal stent serves as a structural support for the polymer member carrying the topoisomerase inhibitor. For example, a polymer-based, drug-containing fiber can be threaded through the stent apertures. The metal stent provides the mechanical support in the vessel after deployment for maintaining vessel patency, and the polymer thread provides a controlled release of the topoisomerase inhibitor. Another example is to provide 10 a drug-loaded polymer sheath for encompassing the stent, as described in U.S. Patent No. 5,383,928 (Scott, *et al.*). Another example is to provide a polymer stent which coexpands with the metal stent when placed in the target vessel, as described in U.S. Patent No. 5,674,242 (Phan, *et al.*).

15 In another embodiment, the stent is formed of a biocompatible polymer, including hydrogels, polyurethanes, polyethylenes, ethylenevinyl acetate copolymers, and the like. One preferred class of polymers are shape-memory polymers, as described for example by Froix, U.S. Patent No. 5,163,952, which is incorporated by reference herein. Stents formed of shape-memory polymers, which include methacrylate-containing and acrylate-containing polymers, readily expand to assume a memory condition to expand and press 20 against the lumen walls of a target vessel, as described by Phan, U.S. Patent No. 5,603,722, which is incorporated by reference in its entirety.

25 In studies in support of the present invention, the topoisomerase inhibitor camptothecin was incorporated into polymer stents and implanted into pig coronary arteries. In other studies, polymer stents containing etoposide, amsacrine, mycophenolic acid, dipyridamole and dexamethasone were prepared and implanted into pig coronary arteries. These studies will now be discussed.

30 The polymer stent used in the studies in support of the invention is illustrated in Figs. 1A-1C. Stent 10 is composed of a unitary strip 12 having two legs 14, 16. The stent typically includes a radio-opaque material, such as gold, stainless steel, platinum, tantalum or metal salts, to provide a means of identifying by x-ray or other imaging technique the location of the stent during and after stent placement. Stent 10 in Fig. 1A includes bands of gold 18, 20 for imaging purposes. The radio-opaque material is incorporated into the stent prior to formation of the polymer or is applied as a coating after formation of the stent.

Fig. 1B shows stent 10 placed in a closed condition for insertion and placement in a target vessel. To place the stent in its small diameter, closed condition, the stent is wound around a cylinder or rod sized according to the diameter of the target vessel. For example, the stent of Fig. 1A can be wrapped around the balloon portion of a balloon catheter and secured thereon by a variety of means, including restraining devices, adhesives or, in the case of memory polymers, by the self-restraining nature of the material. Stent 10 is wound into its closed condition by wrapping legs 14, 16 in the same direction or in opposite directions.

After placement of the stent in a target vessel, the stent is expanded by a stimulus, such as pressure from the balloon portion of the catheter or heat. The leg portions of the stent expand radially until their movement is constrained by the walls of the vessel.

Stents as illustrated in Fig. 1 were prepared as described in Example 1 and were composed primarily of methylmethacrylate and polyethyleneglycol methacrylate. The stents were loaded with camptothecin by applying a concentrated solution of the drug to the surface of the stent.

Camptothecin-containing stents were inserted into the coronary arteries of pigs, as set forth in Examples 2-4. In the study described in Example 2 and 3, a polymer stent was loaded with 1 μ g camptothecin from a solution of the drug in dimethylformamide. The polymer stent was deployed into the pig artery using a balloon catheter. As controls, a commercially available metal stent was inserted and a metal stent carrying a v-shaped polymer stent of the same composition as the test stent, except that it was free of camptothecin.

At the time of insertion of the test and control stents, the coronary artery was characterized using a computer-based coronary angiography analysis system (Umans, V.A., *et al.*, *JACC*, 21(6):1382-1390 (1993)). Boundaries of a selected coronary artery segment were detected automatically from optically magnified and video-digitized regions of interest. The catheter used for insertion of the stents was used as a scaling device to determine the dimensions of the artery at the site of implantation. The original vessel diameter at the time of implantation was determined.

The test stent and the control stents were left in place in the pig artery for one month. The arteries were then explanted from the pig and pressure fixed for morphometric analysis. The minimal lumen diameter of the vessel after the one month treatment period was found by determining the smallest lumen diameter in the region of stent placement. "Late loss" was calculated by subtracting the minimal lumen diameter post treatment from

the minimum inner stent diameter. The percent stenosis was taken as the late loss divided by the original vessel diameter times 100.

5 The camptothecin eluting polymer stent had a late loss of 0.3 mm and a percent diameter stenosis of 10%. The metal stent control had a late loss of 0.49 mm and a percent diameter stenosis of 16%. The metal stent carrying the drug-free polymer stent had a late loss of 1.46 mm and a percent diameter stenosis of 45%. These results are summarized in Tables 2 and 3 in Examples 2 and 3, respectively.

10 In another study, described in Example 4, a camptothecin-eluting polymer stent was prepared by loading the stent with 56 μ g drug from an N-methylpyrrolidone solution. The stent and a metal control stent were inserted into pig coronary arteries, as described above and in Example 4, for a one month period. The coronary arteries were explanted and angiographic images of the arteries with the stents in place were taken.

15 Fig. 2A is a rendering of the angiographic image of the artery treated with the camptothecin-eluting polymer stent of the invention. Fig. 2B shows a rendering of the angiographic image of the artery treated with the metal stent. The narrowing as a result of stenosis in the artery treated with the metal stent (Fig. 2B) is apparent by comparing the figures. Late loss and percent diameter stenosis were determined for the stents and are summarized in Table 4 in Example 4. The metal stent had a late loss of 1.0 mm and a percent diameter stenosis of 38%. The camptothecin-eluting stent had a late loss of 0.5 mm and a percent diameter stenosis of 16%.

20 These studies clearly demonstrate that camptothecin is effective to inhibit the undesirable cell growth associated with vessel injury during coronary angioplasty and stent insertion.

25 In other studies in support of the invention, polymer stents were prepared and loaded with etoposide (Example 5), amsacrine (Example 6) and mycophenolic acid (Example 7). Each of these compounds were successfully loaded into polymer stents.

30 The loading level of drug into the stent can be selected and tailored according to the desired treatment regimen. The loading is readily varied, as will be appreciated by one of skill in the art, by varying the loading solvent, the drug concentration and the polymer composition of the stent. Typically loading levels are between 0.01-50% drug on a weight basis. For camptothecin, the loading level is readily varied between 0.1-10%, with the preferred loading level for effective therapy between about 0.2-9%.

In another embodiment of the invention, a second therapeutic agent is administered along with the topoisomerase inhibitor. The second therapeutic agent, in one embodiment

is radiation, and in another embodiment, is a therapeutic agent which is incorporated into the stent or is administered by another route, such as a systemic or local route of administration described above. Exemplary compounds for use as the second therapeutic agent include paclitaxel, derivatives of paclitaxel including water soluble and non-water soluble derivatives, verapamil, colchicine and dexamethasone.

III. EXAMPLES

The following examples illustrate a method of administering a topoisomerase inhibitor, camptothecin, to a subject, in accordance with the present invention. The examples are in no way intended to limit the scope of the invention.

A. Materials

Camptothecin, etoposide (4'-desmethyllepidophyllotoxin 9-[4,6-O-ethylidene-β-D-glucopyranoside]), amsacrine (4-[9-acridinylamino]-N-[methane-sulfonyl]-m-anisidine), mycophenolic acid (6-[4-hydroxy-6-methoxy-7-methyl-3-oxo-5-phthalanyl]-4-methyl-4-hexanoic acid), dipyridamole and dexamethasone were purchased from Sigma (St. Louis, MO). All solvents were reagent grade.

B. Methods

Late loss was calculated by subtracting the inner diameter of the stent from the measured minimal lumen diameter of the vessel after the one-month treatment period with a stent. Percentage stenosis was taken as the late loss divided by the original vessel diameter x 100.

25

EXAMPLE 1

Polymer Stent Preparation

A polymer stent is prepared according to the procedure described in U.S. Patent No. 5,674,242 (Phan, *et al.*). Briefly, the materials in Table 1 were mixed together in the specified amounts, purged with nitrogen, and then polymerized between glass plates to form thin films having a thickness of approximately 0.14 mm. Prior to polymerization, gold strips were placed at intervals to provide for radio-opacity of the stents.

Table 1

Material	wt%
polyethyleneglycol (1000) methacrylate (NOF Corp.)	32.8
methylmethacrylate (Aldrich)	46.8
butyl methacrylate (Rhom Tech Inc)	14.0
hexanediodimethacrylate (Aldrich)	5.6
Darocur 1173 (Ciba-Geigy) or azobisisobutylnitrile (AIBN, Aldrich)	0.8

5 After polymerization, the film was cut into V-shaped strips (Fig. 1) using a punch and any unpolymerized monomer was removed by solvent extraction.

The selected drug was loaded into the polymer stent by preparing a solution of the drug in a suitable solvent, typically an alcohol (isopropanol or methanol), N-methylpyrrolidone or dimethylformamide. The stent was weighed and placed in a clean 10 container. A known volume of the drug solution was pipetted over the surface of the stent. The stent was then placed in a vacuum oven at about 40 °C for between 1-3 days to dry.

EXAMPLE 2

Camptothecin-Containing Polymer Stent

15 A V-shaped polymer stent was prepared as described in Example 1. The stent was loaded with camptothecin as follows. 5.4 mg camptothecin was dissolved in 27 ml dimethylformamide and 5 μ l of this drug solution was pipetted over the surface of the stent to obtain a loading of 1 μ g in the stent.

20 The camptothecin-containing polymer stent and, as a control, a commercially available metal, corrugated-ring type stent were placed into the coronary arteries of a healthy Domestic Farm Swine pig (Pork Power, Inc.) by conventional techniques using a commercially available catheter (Advanced Cardiovascular Systems). At the time of insertion, the coronary artery was characterized using a computer-based coronary angiography analysis system (Umans, V.A., *et al.*, *JACC*, **21**(6):1382-1390 (1993)).
25 Boundaries of the coronary artery segment were detected automatically from optically magnified and video-digitized regions of interest. The catheter used for insertion of the stents was used as a scaling device to determine the dimensions of the artery at the site of implantation. The original vessel diameter at the time of implantation was determined.

The stents were imaged during and after the insertion procedure to ensure proper placement.

One month after placement of the test stent and the control stent, the pig was euthanized and the heart and coronary arteries explanted. The arteries were pressure fixed 5 for morphometric analysis. The minimal lumen diameter of the vessel after the one month treatment period was found by determining the smallest lumen diameter in the region of stent placement. "Late loss" was calculated by subtracting the minimal lumen diameter post treatment from the minimum inner stent diameter. The percent stenosis was taken as the late loss divided by the original vessel diameter times 100. The late loss and percent 10 stenosis are shown in Table 2.

Table 2

STENT	Late Loss	Percent Stenosis
control, metal stent	0.49 mm	16%
camptothecin polymer stent	0.3 mm	10%

15

EXAMPLE 3
Camptothecin-Containing Polymer Stent

A camptothecin-containing polymer stent was prepared as described in Example 2. The control metal stent was wrapped with a similar, but camptothecin-free V-shaped 20 polymer stent. Both stents were inserted into the coronary arteries of a pig, as described in Example 2 and explanted after one month. The late loss and percent restenosis are shown in Table 3.

25

Table 3

STENT	Late Loss	Percent Stenosis
control: metal stent carrying polymer stent (no drug)	1.46 mm	45%
camptothecin polymer stent	0.3 mm	10%

EXAMPLE 4**Camptothecin-Containing Polymer Stent**

5 A V-shaped polymer stent was prepared as described in Example 1 and was loaded with camptothecin by the following procedure. 50.2 mg camptothecin was dissolved in 4.5 ml N-methylpyrrolidone and 5 μ l of this solution was pipetted over the surface of the stent to obtain a loading of 56 μ g. The stent was dried in a vacuum oven at 40 °C for 3 days.

10 The camptothecin-containing polymer stent and, as a control, a commercially available metal, corrugated ring-type stent were placed into the coronary arteries of a healthy Domestic Farm Swine pig (Pork Power, Inc.) by conventional techniques using a commercially available catheter (Advanced Cardiovascular Systems). The stents were imaged during and after the insertion procedure to ensure proper placement using convention angiographic imaging techniques.

15 One month after placement of the test stent and the control stent, the pig was euthanized and the heart and coronary arteries explanted. The arteries were pressure fixed for morphometric analysis. Images of the coronary arteries containing the polymer camptothecin stent and the control metal stent are shown in Figs. 2A and 2B, respectively. The late loss and percent stenosis are shown in Table 4.

Table 4

20

STENT	LATE LOSS	PERCENT STENOSIS
control: metal stent	1.0 mm	38%
camptothecin polymer stent	0.5 mm	16%

EXAMPLE 5**Etoposide-Containing Polymer Stent**

25 A V-shaped polymer stent was prepared as described in Example 1 and loaded with etoposide as follows. 0.0251 grams of etoposide was weighed into a polypropylene test tube with cap. 500 μ l of dimethylformamide was added and the tube agitated until the etoposide was dissolved.

30 The stent was loaded with etoposide by the method described above in the methods section, by placing 5 μ l over the V-shaped stent and drying the stent in an oven for 24

hours. The loading was 250 $\mu\text{g}/\text{stent}$.

EXAMPLE 6

Amsacrine-Containing Polymer Stent

5 Five V-shaped polymer stents were prepared as described in Example 1. A solution containing 10 mg of amsacrine in 300 μl of dimethylformamide was prepared and mixed until the amsacrine was dissolved.

10 The five stents were loaded with amsacrine by placing 5 μl of the drug solution over the surface of each V-shaped stent and drying the stents in an oven for 24 hours. The loading procedure was repeated two times more to achieve a drug loading of 500 $\mu\text{g}/\text{stent}$.

EXAMPLE 7

Mycophenolic Acid-Containing Polymer Stent

15 Four V-shaped polymer stents were prepared as described in Example 1. A solution containing 53.35 mg of mycophenolic acid in 500 μl of dimethylformamide was prepared and agitated until the mycophenolic acid was dissolved.

20 The four stents were loaded with mycophenolic acid by placing 5 μl of the drug solution over the surface of each V-shaped stent and drying the stents in an oven for 24 hours. The drug loading was about 534 $\mu\text{g}/\text{stent}$.

EXAMPLE 8

Polymer Stent Containing Camptothecin and Dexamethasone

25 A V-shaped polymer stent is prepared as described in Example 1. The stent is loaded with camptothecin and dexamethasone by pipetting 5 μl of a solution containing camptothecin and dexamethasone in dimethylformamide over the stent. The stent is dried in an oven for 24 hours.

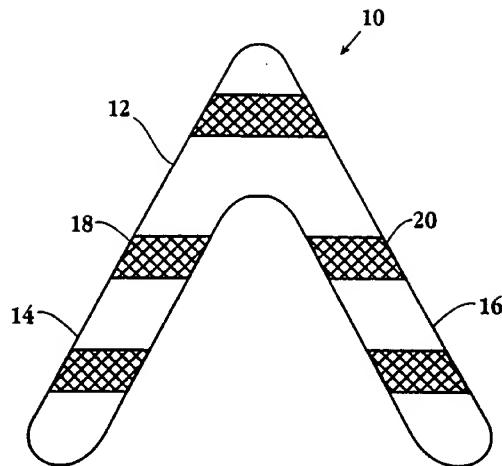
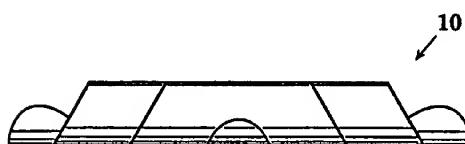
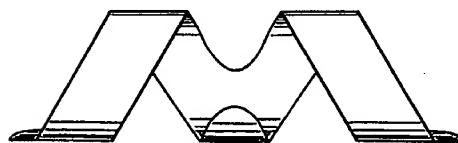
Although the invention has been described with respect to particular embodiments, it will be apparent to those skilled in the art that various changes and modifications can be made without departing from the invention.

IT IS CLAIMED:

1. A composition for use in inhibiting cellular proliferation associated with a hyperproliferative condition in a subject, comprising a therapeutically effective amount of a topoisomerase inhibitor.
5
2. The composition according to claim 1, wherein the hyperproliferative condition is restenosis.
10
3. The composition according to claim 1 or claim 2, wherein the topoisomerase inhibitor is locally delivered via a drug delivery catheter, a guidewire or a stent.
15
4. The composition according to any one of claims 1-3, wherein the topoisomerase inhibitor is selected from the group consisting of camptothecin, irinotecan and topotecan.
5. The composition according to claim 4, wherein the topoisomerase inhibitor is incorporated into a polymer sheath carried on a metal stent.
20
6. The composition according to claim 5, wherein the stent is coated with a synthetic polymer or a biopolymer carrying the topoisomerase inhibitor.
7. The composition according to claim 4, wherein the topoisomerase inhibitor is incorporated into indentations formed on a metal stent.
25
8. The composition according to any of claims 1-7 which further includes a second therapeutic agent.
9. The composition according to claim 8, wherein said second therapeutic agent is a microtubule stabilizing agent.
30
10. The composition according to claim 10, wherein said microtubule stabilizing agent is selected from paclitaxel, derivatives of paclitaxelverapamil, colchicine and dexamethasone.

11. The composition according to claim 8, wherein said second therapeutic agent is radiation treatment.
12. A device for treatment of restenosis, comprising; a stent carrying a 5 therapeutically effective amount of a topoisomerase inhibitor.
13. The device of claim 12, wherein said stent is a polymer stent loaded with a topoisomerase inhibitor selected from the group consisting of camptothecin, irinotecan and topotecan.
14. The device of claim 12, wherein the stent is a metal stent and the topoisomerase inhibitor is incorporated into a polymer sheath carried on the metal stent.
15. The device of claim 12, wherein the stent is coated with a synthetic polymer or a biopolymer carrying the topoisomerase inhibitor.
16. The device of claim 12, wherein the stent is a metal stent and the topoisomerase inhibitor is incorporated into indentations formed in the stent.
20. 17. The device of claim 12, which further includes a second therapeutic agent for treatment of restenosis.
18. The device of claim 17, wherein said second therapeutic agent is a microtubule stabilizing agent.
25. 19. The device of claim 18, wherein said microtubule stabilizing agent is selected from the group consisting of paclitaxel, derivatives of paclitaxel verapamil, colchicine and dexamethasone.
30. 20. Use of a composition according to any of claims 1-11 for the manufacture of a medicament for treatment of restenosis.

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**Fig. 1A****Fig. 1B****Fig. 1C**

2/2

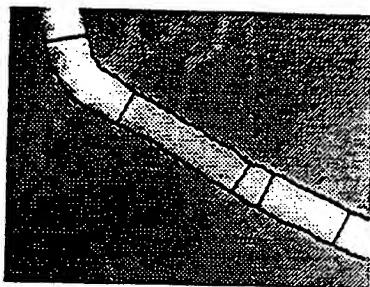


Fig. 2A

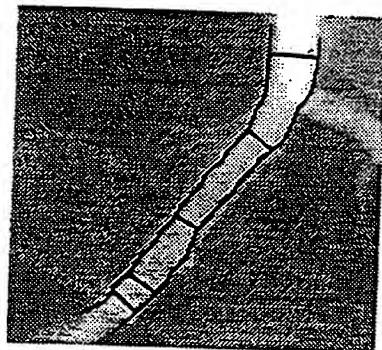


Fig. 2B

INTERNATIONAL SEARCH REPORT

Int'l Application No

PCT/US 99/14386

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61L29/00	A61L31/00	A61K31/47	A61K31/57	A61K31/165
A61K31/275	A61K31/335			

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61L A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 33552 A (LI CHUN ; WALLACE SIDNEY (US); YU DONG FANG (US); WALLACE TECH INC) 18 September 1997 (1997-09-18) abstract page 1, line 4-9 page 3, line 12-25 page 4, line 4-14 page 5, line 4-6 page 6, line 15-27 page 9, line 12-28; example 3 --- -/-/	1-6, 8-10, 12-19

 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

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Date of the actual completion of the international search

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INTERNATIONAL SEARCH REPORT

Int'l. Application No

PCT/US 99/14386

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 17331 A (COOK INC ;MED INST INC (US)) 30 April 1998 (1998-04-30) abstract page 1, line 2-12 page 4 -page 5 page 6, line 1-7 page 7, line 22-30 page 9, line 9,10,28-32 page 10, line 7-13 page 14, line 2-5 page 15, line 6-9 page 16, line 1-4 page 17, line 2,25,26 ---	1-3, 8-11,20
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A	WO 97 31003 A (IST NAZ STUD CURA DEI TUMORI ;MERLINI LUCIO (IT); PENCO SERGIO (IT) 28 August 1997 (1997-08-28) abstract ---	1,4,12, 13
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Information on patent family members

International Application No

PCT/US 99/14386

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US 5569197 A	29-10-1996	CA 2208168 A EP 0799070 A WO 9619255 A JP 10500606 T		27-06-1996 08-10-1997 27-06-1996 20-01-1998



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(21) International Application Number: PCT/US99/14386 (22) International Filing Date: 24 June 1999 (24.06.99)		(81) Designated States: CA, JP, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).	
(30) Priority Data: 60/090,764 26 June 1998 (26.06.98) US		Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
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(74) Agents: MOHR, Judy, M. et al.; Dehlinger & Associates, P.O. Box 60850, Palo Alto, CA 94306-0850 (US).			
(54) Title: TOPOISOMERASE INHIBITORS FOR PREVENTION OF RESTENOSIS			
(57) Abstract			
<p>A method of inhibiting cellular proliferation associated with a hyperproliferative condition, such as restenosis, is described. The method includes administering a topoisomerase inhibitor. A stent for local administration of the topoisomerase inhibitor is also described.</p>			

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Topoisomerase Inhibitors for Prevention of Restenosis

This application claims the priority of U.S. provisional application Serial No. 60/090,764, filed June 26, 1998, which is incorporated herein by reference.

5

Field of the Invention

The present invention relates to a method of inhibiting cellular proliferation in a subject.

10 Background of the Invention

Vascular disease is a leading cause of death and disability in westernized societies. Atherosclerosis is one of the more common forms of vascular disease and leads to insufficient blood supply to critical body organs resulting in heart attack, stroke and kidney failure. Atherosclerosis also causes complications in people suffering from hypertension and diabetes.

15 Atherosclerosis has been described as a form of vascular injury. A normal vessel wall consists of three reasonably well-defined layers: the intima, the media and the adventitia. The intima lines the lumen of all arteries and is composed of a single continuous layer of endothelial cells. The media consists of only one cell type, the smooth 20 muscle cell, arranged in either a single layer or multiple lamellae. These cells are surrounded by small amounts of collagen and elastic fibers. The outermost layer of the artery is the adventitia, which consists of a loose interwoven admixture of collagen bundles, elastic fibers, smooth muscle cells and fibroblasts (Harrison's PRINCIPLES OF INTERNAL MEDICINE, 12th Edition, McGraw-Hill, Inc., 1991, Chapter 195).

25 While the processes causing atherosclerosis are complex and not completely understood, an underlying pathology to the numerous theories for the cause of atherosclerosis is the abnormal migration and proliferation of medial-smooth muscle cells into the intima (Harrison). The proliferation of the smooth muscle cells in the intima ultimately blocks blood flow and makes vessels abnormally susceptible to local blood 30 clotting.

A similar pathology is also implicated in restenosis, the so-called recurrence of stenosis or artery stricture after corrective surgery. In fact, restenosis has been described as an accelerated atherosclerosis induced by injury (Forrester, J.S., *et al.*, JACC, 17(3):758-769 (1991)). Restenosis results from vascular smooth muscle cell proliferation,

migration and neo-intimal accumulation, due to incompletely understood processes involving regulatory molecules, such as platelet derived growth factor (Ferns, G.A., *et al.*, *Science*, 253:1129 (1991)).

Restenosis has been observed to occur after coronary artery bypass surgery, heart 5 transplantation, atherectomy, laser ablation and balloon angioplasty. In particular, restenosis is common after balloon angioplasty, also referred to as percutaneous transluminal coronary angioplasty, which is widely used as a treatment modality in patients with coronary artery disease to reduce lumen obstruction and improve coronary blood flow. It is estimated that between 25-35% of patients develop restenosis within 1-3 months after 10 balloon coronary angioplasty, necessitating further interventions such as repeat angioplasty or coronary bypass surgery.

Therapies to reduce restenosis have focused on administration of chemotherapeutic agents which either interfere with formation of thrombosis or suppress smooth muscle cell proliferation. Anti-coagulants for suppression of thrombosis include heparin, warfarin, low 15 molecular weight heparin and hirudin (Lovqvist, A., *et al.*, *J. Int. Medicine*, 233:215-116 (1993)). Agents for inhibiting the proliferation of smooth muscle cells include glucocorticoids, angiotensin converting enzyme inhibitors, colchicine, vincristine, actinomycin, low molecular weight heparin, platelet derived growth factor and others (Lovqvist, A., *et al.*). More recently, paclitaxel (TAXOL[®]) has been suggested for use in 20 preventing restenosis (Kinsella, J.L. and Sollott, S. J., U.S. Patent No. 5,616,608, issued April 1, 1997).

However, of all the drugs tested, none have been found to be sufficiently effective, and some, such as colchicine, have been reported to be ineffective (O'Keefe, J.H., *et al.*, *JACC*, 19(7):1597 (1992)).

25

Summary of the Invention

Accordingly, it is an object of the present invention to provide a method of inhibiting cellular proliferation associated with restenosis and atherosclerosis.

In one aspect, the invention includes a method of inhibiting cell proliferation 30 associated with a hyperproliferative condition in a subject by administering to the subject a therapeutically effective amount of a topoisomerase inhibitor.

In one embodiment, the topoisomerase inhibitor is selected from the group consisting of camptothecin, irinotecan and topotecan.

In one embodiment, the hyperproliferative condition is restenosis.

In one embodiment, the method of administration is by local administration of the topoisomerase inhibitor. Local administration of the drug is, in one embodiment, by means of a drug delivery catheter, or in another embodiment, by a guidewire.

5 In another embodiment, the topoisomerase inhibitor is locally administered to the treatment site via a stent carrying the topoisomerase inhibitor. The stent, in one embodiment, is a polymer stent loaded with a topoisomerase inhibitor selected from the group consisting of camptothecin, irinotecan and topotecan.

10 In other embodiments, the stent is a metal stent and the topoisomerase inhibitor is incorporated into a polymer sheath carried on the metal stent, or the metal stent is coated with a synthetic polymer or a biopolymer carrying the topoisomerase inhibitor, or the metal stent includes surface indentations in which the topoisomerase inhibitor is incorporated.

15 In another embodiment, the method includes coadministering a second therapeutic agent with the topoisomerase inhibitor. The second therapeutic agent, in one embodiment, is verapamil, dexamethasone or a microtubule stabilizing agent such as paclitaxel, derivatives of paclitaxel, and colchicine. In another embodiment, the second therapeutic agent is radiation therapy.

In another aspect, the invention includes a method of inhibiting restenosis in a patient by administering to the patient an effective amount of a topoisomerase inhibitor.

20 In another aspect, the invention includes a device for treatment of restenosis, comprising a stent carrying a therapeutically effective amount of a topoisomerase inhibitor.

In one embodiment, the stent is a polymer stent loaded with a topoisomerase inhibitor selected from camptothecin, irinotecan and topotecan.

25 In another embodiment, the stent is a metal stent and the topoisomerase inhibitor is incorporated into a polymer sheath carried on the metal stent. In another embodiment, the metal stent is coated with a synthetic polymer or a biopolymer carrying the topoisomerase inhibitor. In a further embodiment, the metal stent has surface indentations and the topoisomerase inhibitor is incorporated into the pits.

30 The stent, in another embodiment, includes a second therapeutic agent for treatment of restenosis. The second therapeutic agent, in one embodiment, is a microtubule stabilizing agent such as paclitaxel, derivatives of paclitaxel, and colchicine or the agent is verapamil or dexamethasone.

These and other objects and features of the invention will be more fully appreciated when the following detailed description of the invention is read in conjunction with the accompanying drawings.

Brief Description of the Drawings

5 Figs. 1A-1C are illustrations of the basic V-shaped stent used in studies in support of the present invention, where the stent is shown unwound (Fig. 1A), wound to a small diameter for insertion in a vessel (Fig. 1B) and in an open, expanded diameter after placement in a vessel (Fig. 1C); and

10 Figs. 2A-2B are artist-renderings of angiogram images of pig coronary arteries one month after insertion of a camptothecin-loaded stent in accordance with the invention (Fig. 2A) or of a metal, control stent (Fig. 2B).

Detailed Description of the Invention

I. Definitions

"Hyperproliferative condition" refers to undesirable cell growth associated with atherosclerosis, restenosis, proliferative vitreoretinopathy and psoriasis. The term is not intended to include cellular hyperproliferation associated with cancerous conditions.

15 "Undesirable cell growth" or "inhibiting undesirable cell growth" refers to unregulated cell division associated with smooth muscle cells or fibroblasts and to the inhibition of such growth.

20 "Topoisomerase inhibitor" refers to any compound that inhibits the action of topoisomerase enzymes, including topoisomerase I and topoisomerase II. Such inhibitors include camptothecin, derivatives and analogs of camptothecin, such as irinotecan, topotecan and 9-amino-camptothecin, etoposide, teniposide, genistein and mitoxantron.

25 "Administering" as referred to herein is intended to include routes of administration which allow the topoisomerase inhibitor to perform its intended function of inhibiting undesirable cell growth. Such administering includes systemic and local or site specific administration by an appropriate route, such as injection (subcutaneous, intravenous, parenteral, intraperitoneal, intrathecal, etc.) oral, inhalation, transdermal, administration by means of a drug delivery catheter, or implantation of a drug-carrying device.

30 "Effective amount" refers to the amount necessary or sufficient to inhibit the undesirable cell growth, *e.g.*, prevent the undesirable cell growth or reduce the existing cell growth. The effective amount can vary depending on factors known to those of skill in the art, such as the type of cell growth, the mode and regimen of administration, the size of the subject, the severity of the cell growth, etc. One of skill in the art would be able to consider such factors and make the determination regarding the effective amount.

"Pharmaceutically acceptable carrier" refers to any substance coadministered with the topoisomerase inhibitor which allows the compound to perform its intended function. Examples of such carriers include solutions, solvents, dispersion media, delay agents, emulsions, microparticles and the like.

5

II. Method of Treatment

In the method of the invention, a topoisomerase inhibitor is administered to a subject at risk of developing or suffering from a hyperproliferative condition. The topoisomerase inhibitor is administered in a therapeutically effective amount by a selected 10 route as will be described.

Preferred topoisomerase inhibitors for use in the method of the invention include camptothecin and analogs thereof. Camptothecin is a pentacyclic alkaloid initially isolated from the wood and bark of *Camptotheca acuminata*, a tree indigenous to China (Wall, M.E. *et al.*, *J. Am. Chem. Soc.*, **94**:388 (1966)). Camptothecin exerts its pharmacological 15 effects by irreversibly inhibiting topoisomerase I, an enzyme intimately involved in DNA replication. Methods for the synthesis of camptothecin and camptothecin analogs are known, and are summarized and set forth in U.S. Patent No. 5,244,903, which is herein incorporated by reference in its entirety.

Analogues of camptothecin include SN-38 ((+)-(4S)-4,11-diethyl-4,9-dihydroxy-20 1H-pyran[3',4':6,7]-indolizino[1,2-b]quinoline-3,14(4H,12H)-dione); 9-aminocamptothecin; topotecan (hycamtin; 9-dimethyl-aminomethyl-10-hydroxycamptothecin); irinotecan (CPT-11; 7-ethyl-10-[4-(1-piperidino)-1-piperidino]-25 carbonyloxy-camptothecin), which is hydrolyzed *in vivo* to SN-38; 7-ethylcamptothecin and its derivatives (Sawada, S. *et al.*, *Chem. Pharm. Bull.*, **41**(2):310-313 (1993)); 7-chloromethyl-10,11-methylene-dioxy-camptothecin; and others (SN-22, Kunimoto, T. *et al.*, *J. Pharmacobiodyn.*, **10**(3):148-151: (1987); DX-8951f and GG-211 Rothenberg, M.L., *Ann. Oncol.*, **8**(9):837-855 (1997)).

Other topoisomerase inhibitors for use in the method of the invention include 4'-30 (acridinylamino)-methanesulfon-m-aniside (amsacrine) and its analogues, such as N-5-dimethyl-9-(2-methoxy-4-methylsulfonylamino)-phenylamino-4-acridinecarboxamide (CI-921, Traganos, F., *et al.*, *Cancer Res.*, **47**(2):424-432 (1987)) and methyl-N-[4-9-acridinylamino)-2-methoxyphenyl]carbamate hydrochloride (m-AMCA, Moreland, N., *et al.*, *Eur. J. Cancer*, **33**(10):1668-1676 (1997)); 6-N-formylamino-12,13-dihydro-1,11-dihydroxy-13-(beta-D-glucopyranosyl)-5H-indolo[2,3-a]pyrrolo[3,4-c]carbazole-5,7(6H)-

dione (NB-506, Kanzawa, F *et al.*, *Cancer Res.*, 55(13):2806-2813 (1995); etoposide (VP-16, Karato, A., *et al.*, *J. Clin. Oncol.*, 11(10):2030-2035 (1993)); m-AMSA (Nakano, H., *Gan To Kagaku Ryoho*, 18(10):1550-1555 (1991)); 6-[[2-(dimethylamino)-ethyl]amino]-3-hydroxy-7H-indeno[2,1-c]quinolin-7-one dihydrochloride, (TAS-103, Utsugi, T., *et al.*, *Jpn. J. Cancer Res.*, 88(10):992-1002 (1997)); azatoxin (Solary E., *et al.*, *Biochem. Pharmacol.*, 45(12):2249-2456 (1993)); 3-methoxy-11H-pyrido[3',4'-4,5]pyrrolo[3,2-c]quinoline-1,4-dione (AzalQD, Riou, J.F., *et al.*, *Mol. Pharmacol.*, 40(5):699-706 (1991)); anthracenyl-amino acid conjugates (ICRF 506, Meikle, I., *et al.*, *Biochem. Pharmacol.*, 49(12):1747-1757 (1995)); and bis(2,6-dioxopiperazine derivatives (ICRF-154, ICRF-193, ICRF-159 and MST-16, Kizaki, H., *et al.*, *Adv. Enzyme Regul.*, 37:403-423 (1997)).

In one embodiment of the invention, the topoisomerase inhibitor administered is the pharmacologically active enantiomer of a camptothecin analogue having a chiral center. The enantiomer can be resolved from the racemic mixture using techniques known to those of skill in the art.

In another embodiment, the topoisomerase inhibitor is camptothecin or an analogue of camptothecin and is administered in combination with a second therapeutic compound selected from paclitaxel, and derivatives of paclitaxel, calcium channel blockers, such as verapamil, and steroidial non-inflammatory agents, such as dexamethasone. When the second therapeutic agent is a calcium channel blocker, it serves to reduce elimination of the first therapeutic agent from the cells. When the second therapeutic agent is an anti-inflammatory agent, it is effective to reduce inflammation at a site of injury.

The therapeutic compounds described above are used in the method of the invention for inhibiting the growth of vascular smooth muscle cells in the vessel. "Inhibiting" as used herein is intended to include reducing, delaying or eliminating undesirable cell growth. With respect to the embodiment of the invention where the undesirable cell growth is associated with restenosis following balloon coronary angioplasty, "reducing" means decreasing the intimal thickening that results from smooth muscle cell proliferation following angioplasty, either in an animal model or in humans. "Delaying" means delaying the time until onset of visible intimal hyperplasia, as observed histologically or by angiographic techniques, following angioplasty. "Eliminating" refers to completely reducing and/or completely delaying intimal hyperplasia in a subject such that sufficient blood flow in the vessel is established and surgical intervention is not necessary.

The topoisomerase inhibitors are administered in accordance with the invention by any route which provides effective therapy for the inhibition of restenosis. Such routes include but are not limited to systemic administration of the drug by injection, including bolus, pulsed and continuous administration injected intravenously, subcutaneously, 5 intramuscularly, intraperitoneally, etc. Continuous or delayed release formulations are also contemplated, both for systemic administration and local or site specific administration.

One preferred mode of administering the topoisomerase inhibitor is via a drug delivery catheter, such as those described in U.S. Patent Nos. 5,558,642, 5,295,962, 10 5,171,217 and 5,674,192. Typically, such catheters have a flexible shaft and an inflatable balloon at the distal end of the shaft. The catheter is inserted into a vessel in an un-inflated condition and the balloon is positioned at the site to be treated with the topoisomerase inhibitor. The balloon member is inflated and apertures in the balloon assembly provide drug carried in the catheter to be delivered to the target site. The drug can be carried in solution form, entrapped in microparticles of a physiologically compatible polymer or 15 incorporated into a polymer, such as a hydrogel, which is coated on the balloon region for rapid release of the drug during expansion of the balloon. Catheters such as these provide a convenient way to administer the drug in conjunction with a balloon angioplasty procedure.

In other embodiments, the topoisomerase inhibitor is administered to the target site 20 by an infusion catheter or by a drug delivery guidewire. An infusion catheter provides delivery of agents to a target site by placing the tip of the catheter at the site and connecting the catheter to a pump. The tip of the catheter generally includes openings through which the agent is pumped at a desired rate to the target site (U.S. Patent No. 5,720,720). A drug delivery guidewire has been described in U.S. Patent No. 5,569,197, where the guidewire 25 is hollow and has an opening at its distal end for infusion of a drug therethrough.

In a preferred embodiment, the topoisomerase inhibitor is administered locally to the site of undesired cell growth in the form of an implanted medical device, such as a stent. Endovascular stents for use following balloon angioplasty are known in the art and described in, for example, U.S. Patent Nos. 5,395,390 (Simon), 4,739,762 (Palma), 30 5,195,984 (Schatz) and 5,163,952 (Froix).

In one embodiment, the stent is a metal stent. Exemplary biocompatible and nontoxic metals for stents include nickel-titanium alloys, tantalum, and steel. In this embodiment, the topoisomerase inhibitor can be adsorbed onto the stent or incorporated into indentations, *i.e.*, pockets, grooves or pits, formed on the surface of the stent. In

another embodiment, the metal stent is coated with a polymer-drug solution containing the selected topoisomerase inhibitor or drug combination by dipping the stent in the solution or spraying the stent with the solution.

5 The metal stent, in other embodiments, is adapted to carry a polymer member, where the metal stent serves as a structural support for the polymer member carrying the topoisomerase inhibitor. For example, a polymer-based, drug-containing fiber can be threaded through the stent apertures. The metal stent provides the mechanical support in the vessel after deployment for maintaining vessel patency, and the polymer thread provides a controlled release of the topoisomerase inhibitor. Another example is to provide 10 a drug-loaded polymer sheath for encompassing the stent, as described in U.S. Patent No. 5,383,928 (Scott, *et al.*). Another example is to provide a polymer stent which coexpands with the metal stent when placed in the target vessel, as described in U.S. Patent No. 5,674,242 (Phan, *et al.*).

15 In another embodiment, the stent is formed of a biocompatible polymer, including hydrogels, polyurethanes, polyethylenes, ethylenevinyl acetate copolymers, and the like. One preferred class of polymers are shape-memory polymers, as described for example by Froix, U.S. Patent No. 5,163,952, which is incorporated by reference herein. Stents formed of shape-memory polymers, which include methacrylate-containing and acrylate-containing polymers, readily expand to assume a memory condition to expand and press 20 against the lumen walls of a target vessel, as described by Phan, U.S. Patent No. 5,603,722, which is incorporated by reference in its entirety.

25 In studies in support of the present invention, the topoisomerase inhibitor camptotheycin was incorporated into polymer stents and implanted into pig coronary arteries. In other studies, polymer stents containing etoposide, amsacrine, mycophenolic acid, dipyridamole and dexamethasone were prepared and implanted into pig coronary arteries. These studies will now be discussed.

30 The polymer stent used in the studies in support of the invention is illustrated in Figs. 1A-1C. Stent 10 is composed of a unitary strip 12 having two legs 14, 16. The stent typically includes a radio-opaque material, such as gold, stainless steel, platinum, tantalum or metal salts, to provide a means of identifying by x-ray or other imaging technique the location of the stent during and after stent placement. Stent 10 in Fig. 1A includes bands of gold 18, 20 for imaging purposes. The radio-opaque material is incorporated into the stent prior to formation of the polymer or is applied as a coating after formation of the stent.

Fig. 1B shows stent 10 placed in a closed condition for insertion and placement in a target vessel. To place the stent in its small diameter, closed condition, the stent is wound around a cylinder or rod sized according to the diameter of the target vessel. For example, the stent of Fig. 1A can be wrapped around the balloon portion of a balloon catheter and secured thereon by a variety of means, including restraining devices, adhesives or, in the case of memory polymers, by the self-restraining nature of the material. Stent 10 is wound into its closed condition by wrapping legs 14, 16 in the same direction or in opposite directions.

After placement of the stent in a target vessel, the stent is expanded by a stimulus, such as pressure from the balloon portion of the catheter or heat. The leg portions of the stent expand radially until their movement is constrained by the walls of the vessel.

Stents as illustrated in Fig. 1 were prepared as described in Example 1 and were composed primarily of methylmethacrylate and polyethyleneglycol methacrylate. The stents were loaded with camptothecin by applying a concentrated solution of the drug to the surface of the stent.

Camptothecin-containing stents were inserted into the coronary arteries of pigs, as set forth in Examples 2-4. In the study described in Example 2 and 3, a polymer stent was loaded with 1 μ g camptothecin from a solution of the drug in dimethylformamide. The polymer stent was deployed into the pig artery using a balloon catheter. As controls, a commercially available metal stent was inserted and a metal stent carrying a v-shaped polymer stent of the same composition as the test stent, except that it was free of camptothecin.

At the time of insertion of the test and control stents, the coronary artery was characterized using a computer-based coronary angiography analysis system (Umans, V.A., *et al.*, *JACC*, 21(6):1382-1390 (1993)). Boundaries of a selected coronary artery segment were detected automatically from optically magnified and video-digitized regions of interest. The catheter used for insertion of the stents was used as a scaling device to determine the dimensions of the artery at the site of implantation. The original vessel diameter at the time of implantation was determined.

The test stent and the control stents were left in place in the pig artery for one month. The arteries were then explanted from the pig and pressure fixed for morphometric analysis. The minimal lumen diameter of the vessel after the one month treatment period was found by determining the smallest lumen diameter in the region of stent placement. "Late loss" was calculated by subtracting the minimal lumen diameter post treatment from

the minimum inner stent diameter. The percent stenosis was taken as the late loss divided by the original vessel diameter times 100.

5 The camptothecin eluting polymer stent had a late loss of 0.3 mm and a percent diameter stenosis of 10%. The metal stent control had a late loss of 0.49 mm and a percent diameter stenosis of 16%. The metal stent carrying the drug-free polymer stent had a late loss of 1.46 mm and a percent diameter stenosis of 45%. These results are summarized in Tables 2 and 3 in Examples 2 and 3, respectively.

10 In another study, described in Example 4, a camptothecin-eluting polymer stent was prepared by loading the stent with 56 μ g drug from an N-methylpyrrolidone solution. The stent and a metal control stent were inserted into pig coronary arteries, as described above and in Example 4, for a one month period. The coronary arteries were explanted and angiographic images of the arteries with the stents in place were taken.

15 Fig. 2A is a rendering of the angiographic image of the artery treated with the camptothecin-eluting polymer stent of the invention. Fig. 2B shows a rendering of the angiographic image of the artery treated with the metal stent. The narrowing as a result of stenosis in the artery treated with the metal stent (Fig. 2B) is apparent by comparing the figures. Late loss and percent diameter stenosis were determined for the stents and are summarized in Table 4 in Example 4. The metal stent had a late loss of 1.0 mm and a percent diameter stenosis of 38%. The camptothecin-eluting stent had a late loss of 0.5 mm and a percent diameter stenosis of 16%.

20 These studies clearly demonstrate that camptothecin is effective to inhibit the undesirable cell growth associated with vessel injury during coronary angioplasty and stent insertion.

25 In other studies in support of the invention, polymer stents were prepared and loaded with etoposide (Example 5), amsacrine (Example 6) and mycophenolic acid (Example 7). Each of these compounds were successfully loaded into polymer stents.

30 The loading level of drug into the stent can be selected and tailored according to the desired treatment regimen. The loading is readily varied, as will be appreciated by one of skill in the art, by varying the loading solvent, the drug concentration and the polymer composition of the stent. Typically loading levels are between 0.01-50% drug on a weight basis. For camptothecin, the loading level is readily varied between 0.1-10%, with the preferred loading level for effective therapy between about 0.2-9%.

In another embodiment of the invention, a second therapeutic agent is administered along with the topoisomerase inhibitor. The second therapeutic agent, in one embodiment

is radiation, and in another embodiment, is a therapeutic agent which is incorporated into the stent or is administered by another route, such as a systemic or local route of administration described above. Exemplary compounds for use as the second therapeutic agent include paclitaxel, derivatives of paclitaxel including water soluble and non-water soluble derivatives, verapamil, colchicine and dexamethasone.

III. EXAMPLES

The following examples illustrate a method of administering a topoisomerase inhibitor, camptothecin, to a subject, in accordance with the present invention. The examples are in no way intended to limit the scope of the invention.

A. Materials

Camptothecin, etoposide (4'-desmethyllepidophyllotoxin 9-[4,6-O-ethylidene- β -D-glucopyranoside), amsacrine (4-[9-acridinylamino]-N-[methane-sulfonyl]-m-anisidine), mycophenolic acid (6-[4-hydroxy-6-methoxy-7-methyl-3-oxo-5-phthalanyl]-4-methyl-4-hexanoic acid), dipyridamole and dexamethasone were purchased from Sigma (St. Louis, MO). All solvents were reagent grade.

B. Methods

Late loss was calculated by subtracting the inner diameter of the stent from the measured minimal lumen diameter of the vessel after the one-month treatment period with a stent. Percentage stenosis was taken as the late loss divided by the original vessel diameter x 100.

25

EXAMPLE 1

Polymer Stent Preparation

A polymer stent is prepared according to the procedure described in U.S. Patent No. 5,674,242 (Phan, *et al.*). Briefly, the materials in Table 1 were mixed together in the specified amounts, purged with nitrogen, and then polymerized between glass plates to form thin films having a thickness of approximately 0.14 mm. Prior to polymerization, gold strips were placed at intervals to provide for radio-opacity of the stents.

Table 1

Material	wt%
polyethyleneglycol (1000) methacrylate (NOF Corp.)	32.8
methylmethacrylate (Aldrich)	46.8
butyl methacrylate (Rhom Tech Inc)	14.0
hexanediodimethacrylate (Aldrich)	5.6
Darocur 1173 (Ciba-Geigy) or azobisisobutylnitrile (AIBN, Aldrich)	0.8

5 After polymerization, the film was cut into V-shaped strips (Fig. 1) using a punch and any unpolymerized monomer was removed by solvent extraction.

The selected drug was loaded into the polymer stent by preparing a solution of the drug in a suitable solvent, typically an alcohol (isopropanol or methanol), N-methylpyrrolidone or dimethylformamide. The stent was weighed and placed in a clean 10 container. A known volume of the drug solution was pipetted over the surface of the stent. The stent was then placed in a vacuum oven at about 40 °C for between 1-3 days to dry.

EXAMPLE 2**Camptothecin-Containing Polymer Stent**

15 A V-shaped polymer stent was prepared as described in Example 1. The stent was loaded with camptothecin as follows. 5.4 mg camptothecin was dissolved in 27 ml dimethylformamide and 5 μ l of this drug solution was pipetted over the surface of the stent to obtain a loading of 1 μ g in the stent.

The camptothecin-containing polymer stent and, as a control, a commercially 20 available metal, corrugated-ring type stent were placed into the coronary arteries of a healthy Domestic Farm Swine pig (Pork Power, Inc.) by conventional techniques using a commercially available catheter (Advanced Cardiovascular Systems). At the time of insertion, the coronary artery was characterized using a computer-based coronary angiography analysis system (Umans, V.A., *et al.*, *JACC*, **21**(6):1382-1390 (1993)). 25 Boundaries of the coronary artery segment were detected automatically from optically magnified and video-digitized regions of interest. The catheter used for insertion of the stents was used as a scaling device to determine the dimensions of the artery at the site of implantation. The original vessel diameter at the time of implantation was determined.

The stents were imaged during and after the insertion procedure to ensure proper placement.

One month after placement of the test stent and the control stent, the pig was euthanized and the heart and coronary arteries explanted. The arteries were pressure fixed 5 for morphometric analysis. The minimal lumen diameter of the vessel after the one month treatment period was found by determining the smallest lumen diameter in the region of stent placement. "Late loss" was calculated by subtracting the minimal lumen diameter post treatment from the minimum inner stent diameter. The percent stenosis was taken as the late loss divided by the original vessel diameter times 100. The late loss and percent 10 stenosis are shown in Table 2.

Table 2

STENT	Late Loss	Percent Stenosis
control, metal stent	0.49 mm	16%
camptothecin polymer stent	0.3 mm	10%

15

EXAMPLE 3
Camptothecin-Containing Polymer Stent

A camptothecin-containing polymer stent was prepared as described in Example 2. The control metal stent was wrapped with a similar, but camptothecin-free V-shaped 20 polymer stent. Both stents were inserted into the coronary arteries of a pig, as described in Example 2 and explanted after one month. The late loss and percent restenosis are shown in Table 3.

Table 3

25

STENT	Late Loss	Percent Stenosis
control: metal stent carrying polymer stent (no drug)	1.46 mm	45%
camptothecin polymer stent	0.3 mm	10%

EXAMPLE 4Camptothecin-Containing Polymer Stent

5 A V-shaped polymer stent was prepared as described in Example 1 and was loaded with camptothecin by the following procedure. 50.2 mg camptothecin was dissolved in 4.5 ml N-methylpyrrolidone and 5 μ l of this solution was pipetted over the surface of the stent to obtain a loading of 56 μ g. The stent was dried in a vacuum oven at 40 °C for 3 days.

10 The camptothecin-containing polymer stent and, as a control, a commercially available metal, corrugated ring-type stent were placed into the coronary arteries of a healthy Domestic Farm Swine pig (Pork Power, Inc.) by conventional techniques using a commercially available catheter (Advanced Cardiovascular Systems). The stents were imaged during and after the insertion procedure to ensure proper placement using 15 convention angiographic imaging techniques.

One month after placement of the test stent and the control stent, the pig was 20 euthanized and the heart and coronary arteries explanted. The arteries were pressure fixed for morphometric analysis. Images of the coronary arteries containing the polymer camptothecin stent and the control metal stent are shown in Figs. 2A and 2B, respectively. The late loss and percent stenosis are shown in Table 4.

Table 4

STENT	Late Loss	Percent Stenosis
control: metal stent	1.0 mm	38%
camptothecin polymer stent	0.5 mm	16%

EXAMPLE 5Etoposide-Containing Polymer Stent

25 A V-shaped polymer stent was prepared as described in Example 1 and loaded with etoposide as follows. 0.0251 grams of etoposide was weighed into a polypropylene test tube with cap. 500 μ l of dimethylformamide was added and the tube agitated until the etoposide was dissolved.

30 The stent was loaded with etoposide by the method described above in the methods section, by placing 5 μ l over the V-shaped stent and drying the stent in an oven for 24

hours. The loading was 250 $\mu\text{g}/\text{stent}$.

EXAMPLE 6

Amsacrine-Containing Polymer Stent

5 Five V-shaped polymer stents were prepared as described in Example 1. A solution containing 10 mg of amsacrine in 300 μl of dimethylformamide was prepared and mixed until the amsacrine was dissolved.

10 The five stents were loaded with amsacrine by placing 5 μl of the drug solution over the surface of each V-shaped stent and drying the stents in an oven for 24 hours. The loading procedure was repeated two times more to achieve a drug loading of 500 $\mu\text{g}/\text{stent}$.

EXAMPLE 7

Mycophenolic Acid-Containing Polymer Stent

15 Four V-shaped polymer stents were prepared as described in Example 1. A solution containing 53.35 mg of mycophenolic acid in 500 μl of dimethylformamide was prepared and agitated until the mycophenolic acid was dissolved.

20 The four stents were loaded with mycophenolic acid by placing 5 μl of the drug solution over the surface of each V-shaped stent and drying the stents in an oven for 24 hours. The drug loading was about 534 $\mu\text{g}/\text{stent}$.

EXAMPLE 8

Polymer Stent Containing Camptothecin and Dexamethasone

25 A V-shaped polymer stent is prepared as described in Example 1. The stent is loaded with camptothecin and dexamethasone by pipetting 5 μl of a solution containing camptothecin and dexamethasone in dimethylformamide over the stent. The stent is dried in an oven for 24 hours.

Although the invention has been described with respect to particular embodiments, it will be apparent to those skilled in the art that various changes and modifications can be made without departing from the invention.

IT IS CLAIMED:

1. A composition for use in inhibiting cellular proliferation associated with a hyperproliferative condition in a subject, comprising a therapeutically effective amount of a topoisomerase inhibitor.
5
2. The composition according to claim 1, wherein the hyperproliferative condition is restenosis.
- 10 3. The composition according to claim 1 or claim 2, wherein the topoisomerase inhibitor is locally delivered via a drug delivery catheter, a guidewire or a stent.
4. The composition according to any one of claims 1-3, wherein the topoisomerase inhibitor is selected from the group consisting of camptothecin, irinotecan and topotecan.
15
5. The composition according to claim 4, wherein the topoisomerase inhibitor is incorporated into a polymer sheath carried on a metal stent.
6. The composition according to claim 5, wherein the stent is coated with a synthetic polymer or a biopolymer carrying the topoisomerase inhibitor.
20
7. The composition according to claim 4, wherein the topoisomerase inhibitor is incorporated into indentations formed on a metal stent.
- 25 8. The composition according to any of claims 1-7 which further includes a second therapeutic agent.
9. The composition according to claim 8, wherein said second therapeutic agent is a microtubule stabilizing agent.
30
10. The composition according to claim 10, wherein said microtubule stabilizing agent is selected from paclitaxel, derivatives of paclitaxelverapamil, colchicine and dexamethasone.

11. The composition according to claim 8, wherein said second therapeutic agent is radiation treatment.
12. A device for treatment of restenosis, comprising; a stent carrying a 5 therapeutically effective amount of a topoisomerase inhibitor.
13. The device of claim 12, wherein said stent is a polymer stent loaded with a topoisomerase inhibitor selected from the group consisting of camptothecin, irinotecan and topotecan.
14. The device of claim 12, wherein the stent is a metal stent and the topoisomerase inhibitor is incorporated into a polymer sheath carried on the metal stent.
15. The device of claim 12, wherein the stent is coated with a synthetic polymer or a biopolymer carrying the topoisomerase inhibitor.
16. The device of claim 12, wherein the stent is a metal stent and the topoisomerase inhibitor is incorporated into indentations formed in the stent.
20. The device of claim 12, which further includes a second therapeutic agent for treatment of restenosis.
18. The device of claim 17, wherein said second therapeutic agent is a microtubule stabilizing agent.
25. The device of claim 18, wherein said microtubule stabilizing agent is selected from the group consisting of paclitaxel, derivatives of paclitaxel verapamil, colchicine and dexamethasone.
30. Use of a composition according to any of claims 1-11 for the manufacture of a medicament for treatment of restenosis.

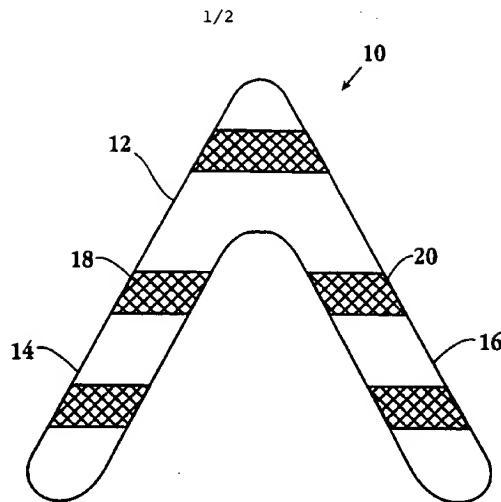


Fig. 1A

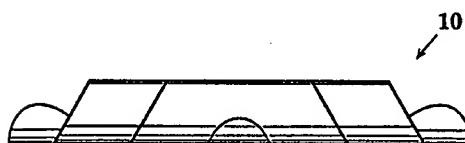


Fig. 1B

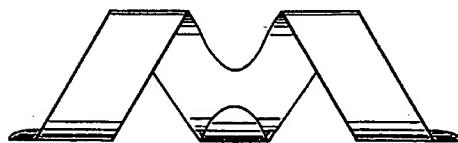


Fig. 1C

2/2

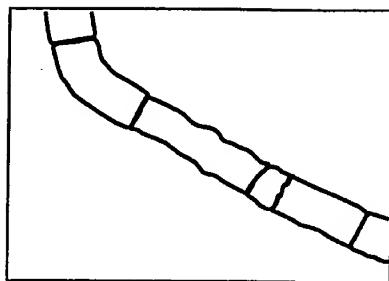


Fig. 2A

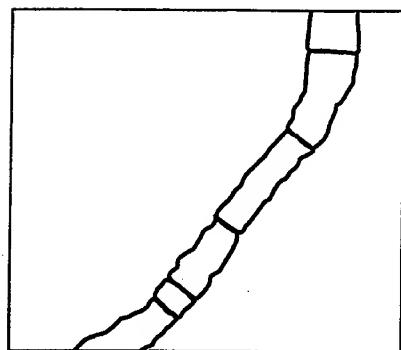


Fig. 2B

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 99/14386

A. CLASSIFICATION OF SUBJECT MATTER					
IPC 6	A61L29/00	A61L31/00	A61K31/47	A61K31/57	A61K31/165
	A61K31/275	A61K31/335			

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61L A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>WO 97 33552 A (LI CHUN ;WALLACE SIDNEY (US); YU DONG FANG (US); WALLACE TECH INC) 18 September 1997 (1997-09-18)</p> <p>abstract</p> <p>page 1, line 4-9</p> <p>page 3, line 12-25</p> <p>page 4, line 4-14</p> <p>page 5, line 4-6</p> <p>page 6, line 15-27</p> <p>page 9, line 12-28; example 3</p> <p>-----</p> <p>-/-</p>	1-6, 8-10, 12-19

 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in that art

"S" document member of the same patent family

Date of the actual completion of the international search

28 October 1999

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INTERNATIONAL SEARCH REPORT

Int'l. Application No

PCT/US 99/14386

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 17331 A (COOK INC ;MED INST INC (US)) 30 April 1998 (1998-04-30) abstract page 1, line 2-12 page 4 -page 5 page 6, line 1-7 page 7, line 22-30 page 9, line 9,10,28-32 page 10, line 7-13 page 14, line 2-5 page 15, line 6-9 page 16, line 1-4 page 17, line 2,25,26 ---	1-3, 8-11,20
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